Epimerization Studies of LSD Using ^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy

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Abstract

A study was conducted to determine the conditions needed to achieve the equilibrium concentration for the epimerization of d-lysergic acid diethylamide (LSD) to iso-LSD. The reaction was followed by integration of the C-9 resonance of LSD and iso-LSD by proton nuclear magnetic resonance (NMR). The C-9 resonance of LSD and iso-LSD appear as singlets at 6.35 and 6.27 ppm respectively. Starting with pure LSD, the conversion to iso-LSD is attained at temperatures above 37°C and pH levels over 7.0. At a pH of 7.0 or higher, the LSD/iso-LSD ratio of 9:1 is achieved after one week at 45°C or two weeks at 37°C. Starting with iso-LSD, the conversion to LSD requires more vigorous conditions. The 9:1 LSD/iso-LSD ratio is attained only after 6 weeks at a temperature of 45°C and a pH of 9.7. At lower pH levels, the reaction proceeds more slowly. The 9:1 LSD/iso-LSD ratio is achieved whether the starting material is LSD or iso-LSD and therefore represents an equilibrium concentration (K = 9). In addition, the more vigorous conditions needed to achieve equilibrium of iso-LSD to LSD demonstrate the difficulty in extraction of the epimerizable proton of iso-LSD. This study is the first to quantitate the epimerization of LSD by NMR techniques and establishes the conditions needed to induce epimerization in solution.

Introduction

The detection and confirmation of d-lysergic acid diethylamide (LSD) in urine still remains a challenge to the toxicology community (1–8). It is generally taken in doses of less than 100 µg and is excreted within 36 h of the initial dose. The drug is extensively metabolized, and less than 1% of the parent compound appears in human urine. Immunoassays designed for LSD detection in urine cross-react with a wide range of metabolites, many of which have not yet been identified (9–14).

In addition, an isomer of LSD, iso-LSD, which appears as an impurity in LSD preparations, is commonly detected in urine. This isomer is found in a high percentage of LSD-containing urine samples, sometimes at higher concentrations than LSD itself (4,12). The isomerization is the result of hydrogen epimerization at the C-8 position of LSD (Figure 1) (15,16). The epimerization reaction is easily followed by observation of the olefin C-9 proton in nuclear magnetic resonance (NMR) spectroscopy.

In order to increase the confirmation rate of LSD in urine, it has been suggested that urine samples be exposed to basic conditions and heat (17). The use of basic treatment has been shown to convert the majority of iso-LSD to LSD (15). This would serve to increase the concentration of LSD in samples in which the LSD/iso-LSD ratio is less than 9:1 for gas chromatography–mass spectrometry (GC–MS) analysis and thereby increase the number of confirmed LSD samples.

In the course of synthesis of LSD from pure d-lysergic acid and diethyl amine, the LSD/iso-LSD epimeric ratio of the resulting purified mixture was consistently 9:1 as determined by NMR (18–22). Because the epimerization reaction is easily followed by NMR, the conditions for the conversion of pure LSD to iso-LSD and iso-LSD to LSD can be easily monitored. The objective of this study was to determine the conditions that cause the epimerization of LSD to occur and to determine if this 9:1 ratio represented an equilibrium concentration.

Experimental

General

All solvents were obtained from Fisher Scientific (Springfield, NJ) unless stated otherwise. Flash-grade silica gel was obtained from EM Science (Gibbstown, NJ). d-Lysergic acid was obtained from Sigma (St. Louis, MO), and all buffers were obtained from Fisher. Carbonyl diimidazole and diethylamine were obtained from Fluka (Ronkonkoma, NY). Specific rotation of LSD was measured with a Perkin-Elmer (Norwalk, CT) model 283 polarimeter using a 10-cm cell at room temperature. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at

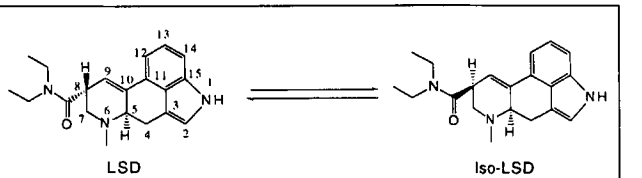

Figure 1. Epimerization of LSD to iso-LSD.

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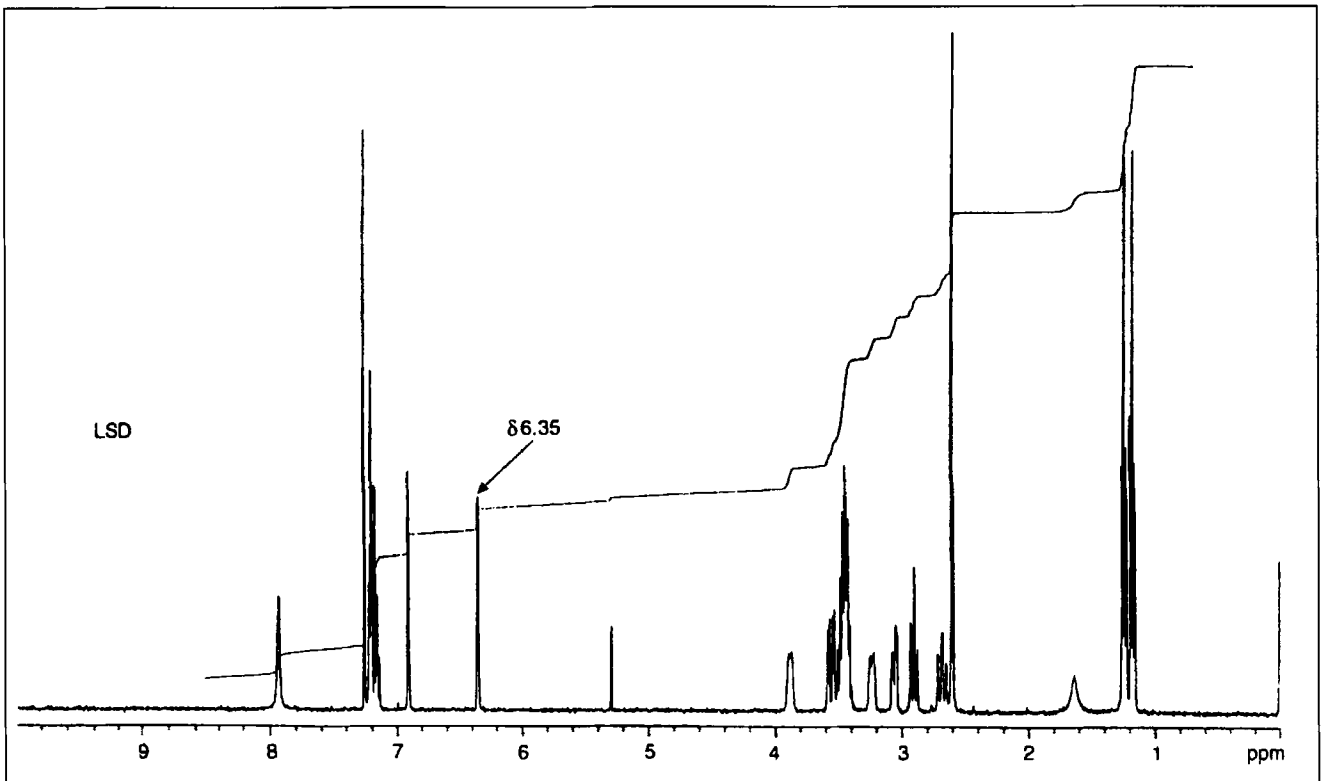

Figure 2. 400 ^{1}H MHz NMR spectrum of LSD.

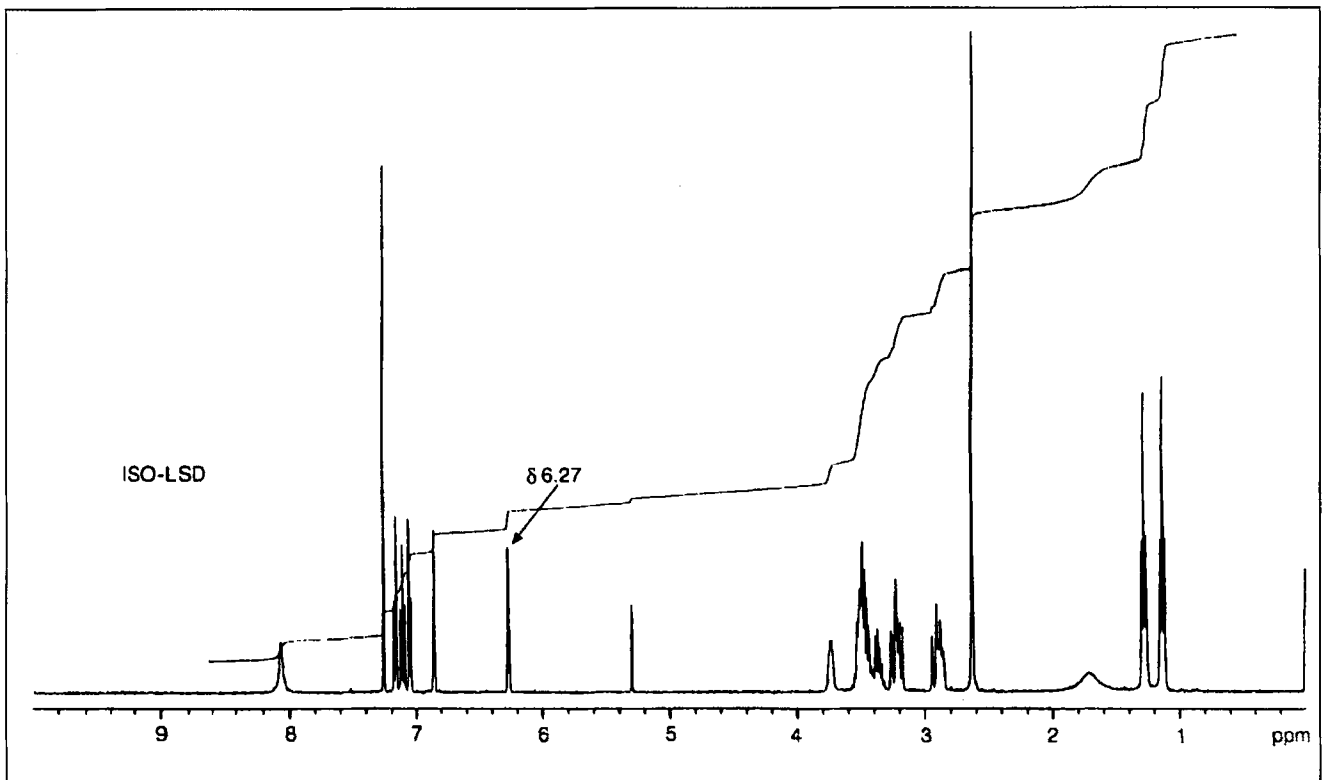

Figure 3. 400 ^{1}H MHz NMR spectrum of iso-LSD.

200 MHz or 400 MHz on either a Varian XL-200 or an XL-400 (Palo Alto, CA) spectrometer respectively using $CDCl_3$ as the solvent. Coupling constants are given in Hertz (Hz). The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. The C-9 proton at δ 6.27 (singlet) for iso-LSD and δ 6.35 (singlet) for LSD was used to determine the extent of epimerization by integrating the area under the peaks (n = 2). NMR spectrum of a known mixture of LSD to iso-LSD at an exact molar ratio of 9:1 was taken (n = 4). Integration of the area of interest was recorded (n = 5) and compared with the theoretical molar ratio of the isomeric mixture. An accuracy of integration was found to be within ± 3% of the theoretical value.

Synthesis of lysergic acid diethyl amide and iso-lysergic acid diethylamide

A mixture of 10.0 g (0.037 mol) of d-lysergic acid (verified by polarimeter measurement, $[\alpha]_D = +42^0$) and 500 mL of dry dimethyl formamide (DMF) under argon was treated with 9.0 g (0.055 mol) of carbonyl diimidazole and stirred at room temperature for 1 h. The reaction was then treated with 38 mL (0.375 mol) of diethylamine and stirred at room temperature overnight. The reaction mixture was concentrated at reduced pressure. The resulting residue was taken up in 500 mL of CH_2Cl_2 and washed with 500 mL of H_2O. The insoluble material was removed by filtration, and the layers were separated. The organic portion was washed with 500 mL of saturated brine solution, dried over anhydrous Na_2SO_4, and concentrated at reduced pressure. The residue was chromatographed on 800 g of silica gel using 3% CH_3OH in CH_2Cl_2 as an eluent to yield 9.0 g (75%) of LSD as a light brown amorphous solid. iso-LSD was eluted with a gradient of 10–20% CH_3OH in CH_2Cl_2 to yield 750 mg of a dark amorphous solid. This was rechromatographed on 150 g of silica gel using 20% CH_3OH in CH_2Cl_2 as an eluent to yield 480 mg of iso-LSD. (LSD)$[\alpha]_D$ = $+50.4^0$ (1%; $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (3H, t, J = 7.1), 1.25 (3H, t, J = 7.1), 2.61 (3H, s), 2.66–2.72 (1H, m), 2.89–2.93 (1H, m), 3.04–3.09 (1H, m), 3.21–3.28 (1H, m), 3.40–3.50 (4H, m), 3.52–3.59 (1H, m), 3.87–3.93 (1H, m), 6.35 (1H, s), 6.92 (1H, s), 7.14–7.24 (3H, m), 7.98 (1H, br s); (iso-LSD): $[\alpha]_D$ = +226.0 (1%; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (3H, t, J = 6.9), 1.28 (3H, t, J ≈ 6.8), 2.63 (3H, s), 2.83–2.95 (2H, m), 3.17–3.28 (2H, m), 3.32–3.42 (1H, m), 3.42–3.57 (4H, m), 3.74 (1H, br s), 6.27 (1H, s), 6.86 (1H, s), 7.04–7.18 (3H, m), 8.07 (1H, br s) (20).

General procedure for epimerization of LSD and iso-LSD

A solution of 12 mg of LSD or iso-LSD in 1.5 mL of absolute ethanol was treated with 5 mL of 50mM potassium biphthalate buffer solution (pH 5) or 5 mL of 50mM potassium phosphate buffer solution (pH 6, 7, 8, or 9) and incubated at 37 and 45°C in the dark for 1, 2, 3, and 6 weeks. The mixture was extracted with 2 × 10 mL of CH_2Cl_2, dried over anhydrous sodium sulfate, and concentrated at reduced pressure to provide a solid residue. (Note: For removal of potassium biphthalate, the CH_2Cl_2 layer was washed with saturated sodium bicarbonate and water before drying over anhydrous sodium sulfate).

Results

A mixture of LSD and iso-LSD was produced by the reaction of diethylamine and d-lysergic acid under basic conditions. The isomers are easily separated on a silica gel chromatographic column. LSD is less polar than iso-LSD and was eluted from the column first. The iso-LSD was then eluted using more polar solvent conditions. The ratio of LSD to iso-LSD was 9:1. Because the lysergic acid starting material was the pure d form, LSD would be expected to be the only product if epimerization were not occurring. The observed 9:1 mixture indicated that epimerization had occurred either at the reaction stage or at column purification. This reaction has been performed several times and has consistently produced an LSD/iso-LSD ratio of 9:1.

The proton NMR spectra of LSD and iso-LSD are shown in Figures 2 and 3 respectively. The resonance of the C-8 proton of LSD appears as a broad multiplet at 3.90 ppm, and the C-8 proton of iso-LSD appears as a broad singlet at 3.74 ppm. When LSD and iso-LSD are mixed together, the NMR resonances of the C-8

Table I. Epimerization LSD to iso-LSD at Various Temperature, Time, and pH

pH observed	1 week at 45°C	1 week at 37°C	2 weeks at 37°C	3 weeks at 25°C
6.4	100:0	100:0	100:0	100:0
7.0	90:10	95:5	90:10	100:0
7.8	90:10	95:5	90:10	100:0
8.5	90:10	95:5	90:10	100:0
9.7	90:10	95:5	90:10	100:0

Table II. Epimerization iso-LSD to LSD at 45°C, Time, and pH (LSD/iso-LSD ratio)

pH observed	1 week	3 weeks	6 weeks
6.4	1:99	3:97	5:95
7.0	12:88	21:79	35:65
7.8	33:67	54:46	70:30
8.6	41:59	68:32	84:16
9.7	52:48	77:23	87:13

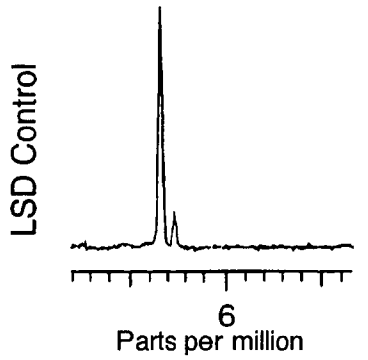

Figure 4. 200 ^{1}H MHz NMR spectrum of LSD incubated at 45°C for 6 weeks at pH 9.7.

protons of both compounds overlap and become broad. The lack of resolution of the C-8 protons in this mixture precludes the use of these resonances to quantitate differences in the epimeric ratios of LSD and iso-LSD.

The C-9 protons of LSD and iso-LSD appear as singlets at 6.35 ppm and 6.27 ppm, respectively (Figures 2 and 3). These proton resonances are easily distinguished in a mixture of LSD and iso-LSD and readily allows for the epimeric quantitation of each compound. Because of the improved resolution of the C-9 protons over the C-8 protons in a LSD/iso-LSD mixture, the C-9 proton resonances were used to follow the course of epimerization.

The epimerization study was conducted using the purified forms of both LSD and iso-LSD. Each compound was dissolved in a buffered aqueous solution containing enough ethanol to solublize all of the compound. The epimerization reaction was studied at measured pH values of 6.4, 7.0, 7.8, 8.5, and 9.7 with temperatures at 25, 37, and 45°C. Read points were 1, 2, 3, and 6 weeks.

Table I shows that epimerization of LSD did not occur at pH levels below 7.0 at any temperature or at 25°C at any pH. At pH levels of 7.0 and higher, the epimerization reaction readily proceeded at 37°C after two weeks and at 45°C after one week. The LSD/iso-LSD equilibrium epimeric ratio in the reactions was 9:1 for all of the conditions where epimerization occurred. The differences of 91 to 9 or 92 to 8 are within the experimental error of the NMR integration (± 3%). Figure 4 shows the 6-week time point for the epimerization of LSD to iso-LSD at pH 9.7 and 45°C. The epimeric ratio of 9:1 is achieved after 1 week and the ratio did not change over the 6-week period.

In studying the epimerization of iso-LSD to LSD, the 45°C temperature was chosen since the reaction of LSD to iso-LSD readily occurred at this temperature after one week. Table II shows that epimerization of iso-LSD did not occur at pH 6.4, but did proceed, albeit more slowly, at pH levels of 7.0 and higher. After six weeks at pH 9.7, the 9:1 ratio was reached, whereas at pH 7.0 and 7.8, the ratio was 35:65 and 70:30, respectively. The data indicate that, compared with LSD, the epimerization of iso-LSD

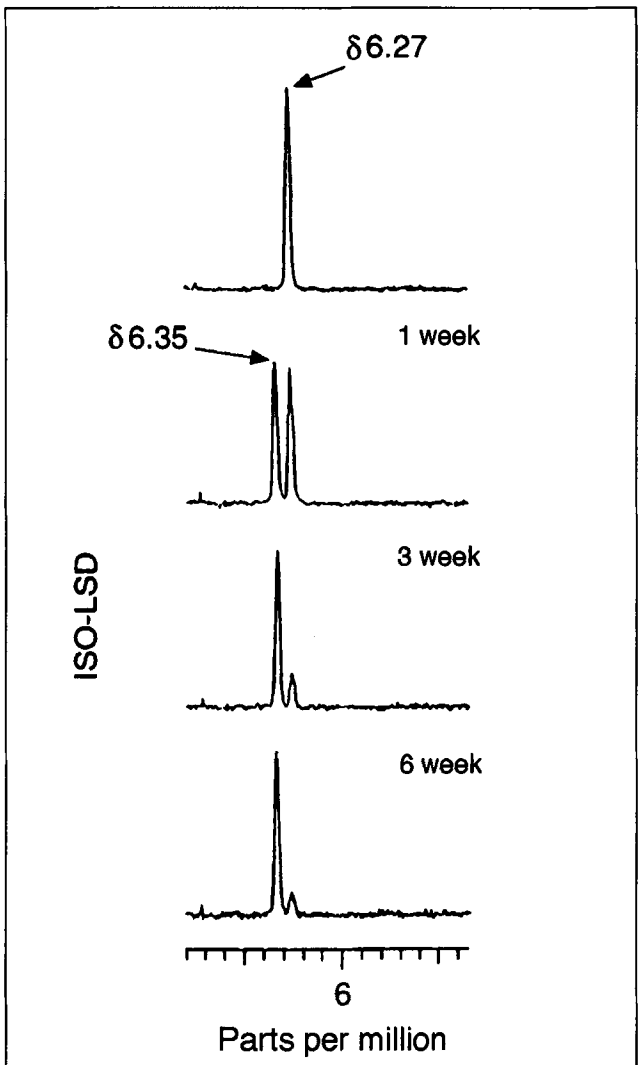

Figure 5. 200 ^{1}H MHz NMR spectra comparison on epimerization of iso-LSD to LSD at 45°C, pH 9.7, conducted at various time intervals.

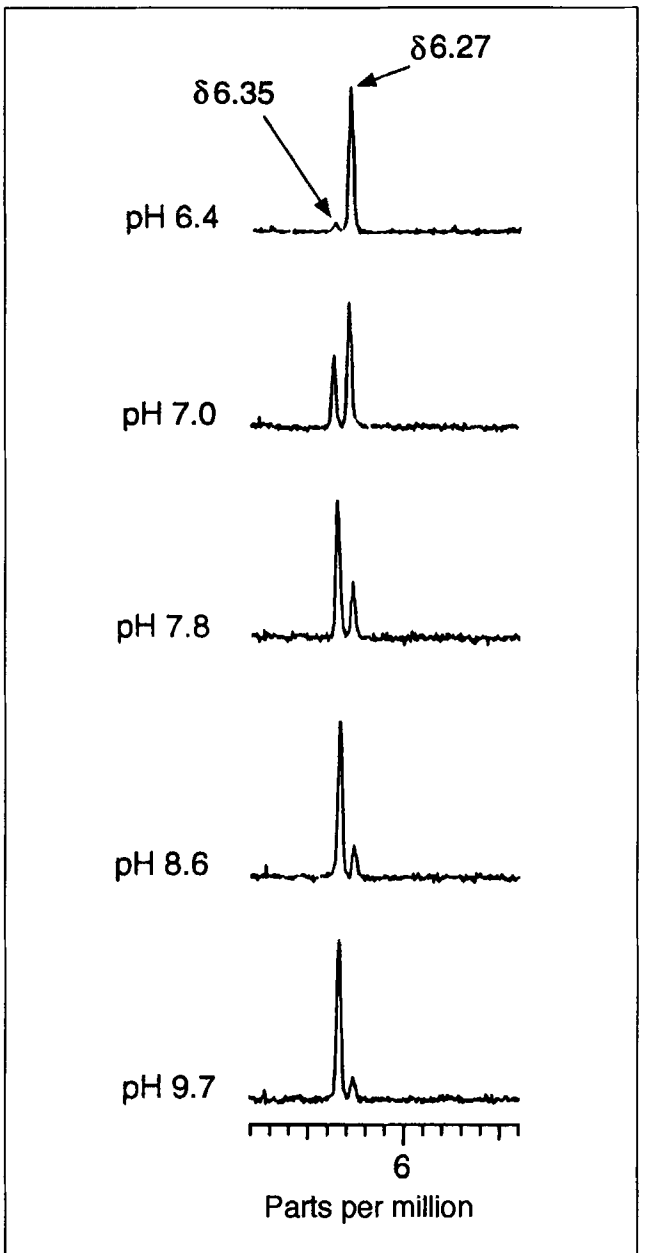

Figure 6. 200 ^{1}H MHz NMR spectra comparison on epimerization of iso-LSD to LSD at 45°C conducted in various pH buffers for 6 weeks.

occured more slowly. In order to achieve the 9:1 LSD/iso-LSD ratio starting with iso-LSD, the reaction took six weeks, which is at least six times slower than the epimerization of LSD to iso-LSD. Epimerization of iso-LSD to LSD was also dependent on pH.

The time course of the reaction of iso-LSD to LSD at the more vigorous condition (pH 9.7 and 45°C) is shown in Figure 5. This figure demonstrates that after one week the ratio of LSD to iso-LSD was 52:48, after three weeks the ratio was 77:23, and after six weeks the ratio approached 9:1. The epimerization of iso-LSD at 45°C after 6 weeks and different pH levels is shown in Figure 6. This figure demonstrates that, at pH levels of 7.0 and higher, the reaction is slow and only at pH levels 8.6 and higher the 9:1 ratio can be achieved.

Conclusion

Epimerization ratios of LSD and iso-LSD can be easily followed using NMR techniques provided that milligram quantities are available. The C-9 proton of LSD and iso-LSD are easily differentiated and integrated.

This study confirms that the 9:1 LSD/iso-LSD ratio observed in the synthesis of LSD is an equilibrium ratio. This was demonstrated by convergence to the same 9:1 LSD/iso-LSD epimer ratio starting with either pure LSD or pure iso-LSD. This 9:1 ratio corresponds to an equilibrium constant of 9 and represents a difference in the free energy of the two epimers of approximately 1.3 kcal/mole (23). In constructing molecular models of each epimer, the diethylamide group is in the more stable equatorial position in LSD and is in the less stable axial position in iso-LSD. The difference of 1.3 kcal/mole between the epimers can be rationalized in terms of the position of the diethylamide.

The epimerization of LSD was previously studied, and the equilibrium ratio was semiquantitatively determined to be 85:15 by paper chromatography (15). The reported study epimerized LSD using a basic anion exchange resin in methanol. The objectives of our study were to determine the epimerization ratio of LSD and iso-LSD in an aqueous solution and to quantitate the epimerization using NMR. The differences between 9:1 and 85:15 can be attributed to the experimental conditions used to epimerize the compounds and the semiquantitative nature of the detection system used in the earlier study.

The results also show that the epimerizable proton on LSD is more easily removed than the same proton of iso-LSD. This is shown by the difference in the amount of time it takes to achieve equilibrium using LSD or iso-LSD. Tables I and II show that under identical conditions the iso-LSD takes at least six times longer to achieve equilibrium and only at the higher pH levels.

Because LSD is not converted to iso-LSD in the body (24), the appearance of iso-LSD in urine samples reflects the LSD/iso-LSD ratio of the drug preparation. Although some LSD may be converted to iso-LSD in basic urine, the maximum amount that can be attributed to this reaction is 10% because the equilibrium lies in the direction of LSD. Samples containing larger amounts of iso-LSD reflect the state of purity of the drug preparation before ingestion. The synthetic procedures used to produce LSD starting with d-lysergic acid would yield largely LSD and would not account for what is seen clinically in certain specimens. The appearance of low LSD/iso-LSD epimer ratios may reflect a reaction that occurs when LSD is placed on a chiral binding surface, such as blotter paper or the starch binders of a tablet, to reflect a single dose. The conditions on the blotter paper may favor the iso-epimer after long-term storage and thus change the LSD/iso-LSD ratio. This process is difficult to envision because the binding materials are neutral and base is needed to extract the epimerizable proton.

Treatment of urine samples, containing low LSD/iso-LSD epimer ratios with base and high temperatures will convert the majority of iso-LSD to LSD and increase the confirmation rate. This procedure, however, would not help to increase the confirmation rate in cases when pure LSD or high LSD/iso-LSD epimer ratio preparations are ingested. Because the epimerization reaction favors LSD, the extent to which iso-LSD appears in urine samples is perplexing. Further studies as to the origin of low LSD/iso-LSD epimer ratios are needed to clarify this issue.

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